Some Observations on a Flagellate of the Genus Cercomonas.

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With 19 Text-figures.

In the present paper I shall describe a flagellate of the genus Cercomonas, a genus first created by Dujardin, in his 'Historie Naturelle des Zoophytes Infusoires,' published in 1841. Since Dujardin's original description numerous flagellates have incorrectly been attributed to this genus, so much so that Klebs, in his 'Flagellatenstudien' (1893), says that this genus has not been defined with sufficient accuracy, that it has been confused with Heteromila and Bodo by the overlooking of the tail flagellum, and that the genus Cercomonas must be rejected. It is undoubtedly true that the genus Cercomonas is very confused, and this confusion has been considerably heightened by the description of Cercomonas from the intestine of man and other animals. Davaine (1854) was the first to record the presence of Cercomonas in the evacuations of a man suffering from cholera. Without going into the question of the correctness or otherwise of Davaine's conclusions, it is undoubtedly a fact that many observers, noting the presence of active flagellates in the intestinal contents, have attributed them at once to the genus Cercomonas, and as a result of this various species of Trichomonas, Lamblia, and possibly other flagellates have been included in this genus. In the present instance the flagellate to be

described was found in the fæces of a patient in the Albert Dock Hospital at the London School of Tropical Medicine. This patient was infected with Entamœba coli, and in order to observe changes in the encysted forms of this amœba some of the fæces were placed in a clean glass-stoppered bottle. In the course of a few days it was noticed that large numbers of flagellates were present. It is probable they had developed from cysts which must have been present in the fæces. On first examination it was seen that these flagellates corresponded very closely with the original description of Dujardin for the genus Cercomonas, and for this I took them to be. On more careful examination I found that the tapering posterior end was in reality a second flagellum, and that this could be traced along the surface of the body to which it was attached as far as the insertion of the long anterior flagellum. The presence of this posterior flagellum and its attachment to the body required very careful observation to make out, for it can only be clearly seen in certain portions of the animal, and it is quite conceivable, as Klebs maintains, that Dujardin overlooked this posterior flagellum. Dujardin's original description of the genus is as follows:

"Genre Cercomonas.

"An. arrondi ou discoïde, tuberculeux, avec un prolongement postérieur variable, en forme de queue, plus ou moins long, plus ou moins filiforme.

Les Cercomonas ne different absolument des Monads que par un prolongement postérieur, formé par la substance même du corps qui s'agglutine au porte-objet, et s'étire plus ou moins, de manière à n'être tantôt qu'un tubercule aminci, tantôt une queue allongée transparente, tantôt enfin un filament presque aussi fin que le filament antérieur, et susceptible d'un mouvement ondulatoire; mais bien souvent j'ai cru voir les Monades passer par degrés l'état de Cercomonas."

A comparison of this description with that now to be given will show how closely the two agree.

The occurrence of this flagellate has been described above. By transplanting into other media I have been able to keep

cultures of this flagellate free from other Protozoa for about a year, and it is only that circumstances preventing me from continuing these observations I now describe what results I have already obtained.

Method of Observation.

I have found the best liquid culture medium to be hay infusion to which a small quantity of fæces has been added. The flagellates will live and multiply in hay infusion alone, but, as in other thin media, the numbers of flagellates are always very small, so that any observation is difficult to make. In the thicker medium the numbers are not only larger but the movements of the flagellates are slower and accordingly more easily followed. For keeping stock cultures small test-tubes were used as in bacteriological methods, but for making observations hanging-drops in the moist chambers of Max Schultze were most useful. In these hanging-drop preparations the flagellates would live for weeks, till finally, all nutriment being used up, encystment followed. By the addition of fresh nutriment to the hanging-drop the culture would commence again.

In addition to the liquid medium I have found the solid agar medium used for the culture of amœbæ most useful. It was first employed for the culture of flagellates by Berliner. This observer, working with Copromonas major, found that on the solid medium the flagellates multiplied rapidly till enormous numbers were present. I can fully confirm this, and for the study of the details of nuclear division the presence of such large numbers of dividing forms is very useful. The medium I employed differed slightly from that used by Berliner. For the culture of amœbæ I have used with success the medium first invented by Musgrave and Clegg, and I have found it equally good for the flagellates at present under discussion. I have employed it in the ordinary Petri dishes. By unveiling the dishes the progress of the culture may be watched under the low powers of the microscope. A very useful method for the

use of this medium, and one which will allow observations to be made with high powers, is the following: A long cover-glass (1½ inches) is taken and carefully cleaned. On a clean slide ridges of Czokor's wax, first recommended to me by Professor Minchin, are so arranged, about an eighth of an inch high, that the cover-glass will form the lid of a box. Some of the medium is melted by placing the test-tube in boiling water, and a small drop of this is allowed to fall on to the cover glass, which is lying on the top of the hot-water oven. By careful tilting of the cover-glass the melted medium will form a very thin layer over the cover-glass, which is then removed so that the medium may solidify. The surface of the medium is then inoculated with a small quantity of material from a previous culture and the cover-glass inverted on the wax ridges. By means of a hot wire and more wax the whole may be completely sealed up. It is most essential that not the smallest opening be left, or it will be found that the medium will quickly dry and the culture end.

In this way it is easy to follow the multiplication of the flagellates with the $\frac{1}{6}$ in. objective, and if the film of medium has been made sufficiently thin the oil-immersion may be employed.

In every case where the flagellates grow in the solid medium their chief nourishment seems to be the numerous bacteria that grow at the same time.

For studying the flagellates in the fixed and stained condition the cover-glass method has been mostly used. Some of the liquid medium or some of the culture scraped from the surface of the agar is spread on a clean cover-glass, and without allowing it to dry it is dropped, film side down, on to the surface of some fixing fluid. Another method of obtaining a film from the agar cultures is this: A cover-glass is dropped on to the surface of the agar culture in a Petri dish. It is gently pressed down till its surface is seen to have touched the culture. On raising it with a needle it will be found that a layer of the culture is adherent to the cover-glass, and it may be fixed as before.

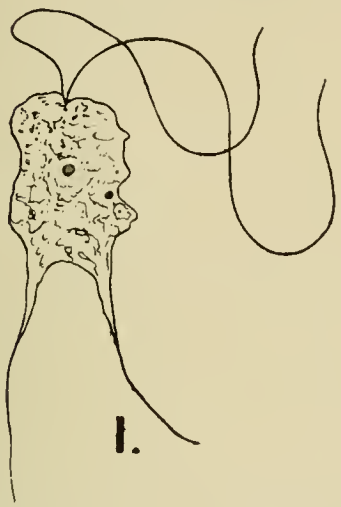
For fixing the flagellates the most useful fixative has been Schaudinn's mixture of two thirds saturated aqueous solution of sublimate and one third alcohol, slightly acidified with acetic acid. This has been used in the manner just described by Schaudinn or in a slightly modified form. The films are best stained with iron-hæmatoxylin.

Description of the Living Flagellates.

When examined in a drop of liquid medium on a slide the

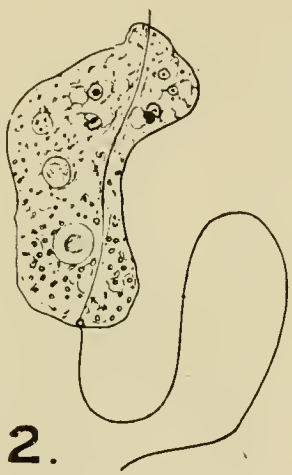
Text-figs. 1–8.—Drawings from life.

Text-fig. 1.



Amœboid form in early division stage.

Text-fig. 2.

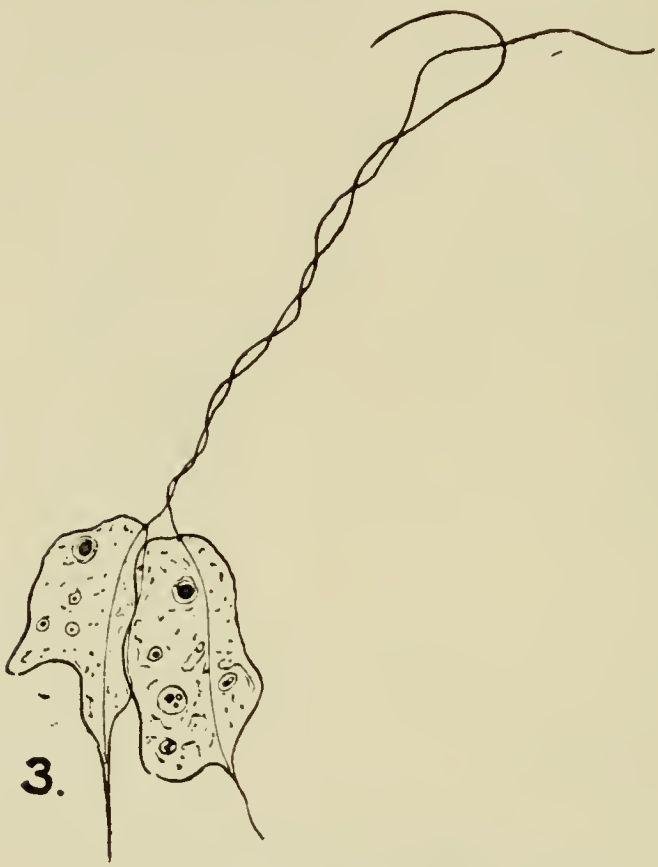


Amœboid form.

flagellates appear as pear-shaped organisms, with a long flagellum, about twice the length of the body, arising from the blunt end. The posterior end of the body is, as a rule, drawn out to a fine and tapering point. By the constant lashing of this long anterior flagellum the animal is drawn along. Sometimes the flagellum is, as it were, hooked around some distant object, and by its flexion pulls the body towards this point. The posterior end of the body, which, as stated above, is also a flagellum, moves much less vigorously than the anterior. Its movements may be quite passive, being only the accidental changes in position produced by the changes

in shape of the body. At other times there is a distinct to-and-fro or lashing movement, but at its maximum it is much less violent than that of the long anterior flagellum. The protoplasm of the body may be continued along this posterior flagellum for a considerable distance. On very careful focussing it can be seen that the posterior flagellum

TEXT-FIG. 3.



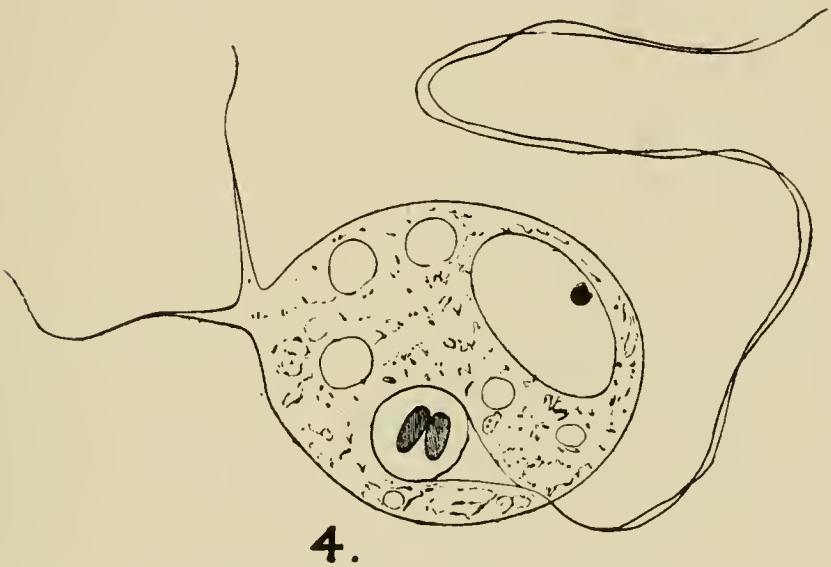
Two amœboid forms with entangled flagella.

is attached to one side of the body, and really arises from the insertion of the anterior flagellum. This is very well shown in some of the figures, e.g. 3, 5, 9. When the body is viewed in certain positions it is seen that it is distinctly flattened along the line of attachment of the posterior flagellum (fig. 10), and when the posterior flagellum is moving at its maximum rate this flattened edge of the body shows slight but distinct undulatory movement, reminding one most strikingly of the

movements of the blood inhabiting Trypanoplasma. Indeed, this flagellate in many respects occupies a position intermediate between the genus Bodo and Trypanoplasma.

The nucleus is clearly visible in the living animal. There is a distinct membrane, and at the centre of the nucleus is a large karyosome. The nuclear membrane is drawn out at one pole towards the insertion of the two flagella, and occasionally a clear line may be detected connecting the apex of

TEXT-FIG. 4.



4.

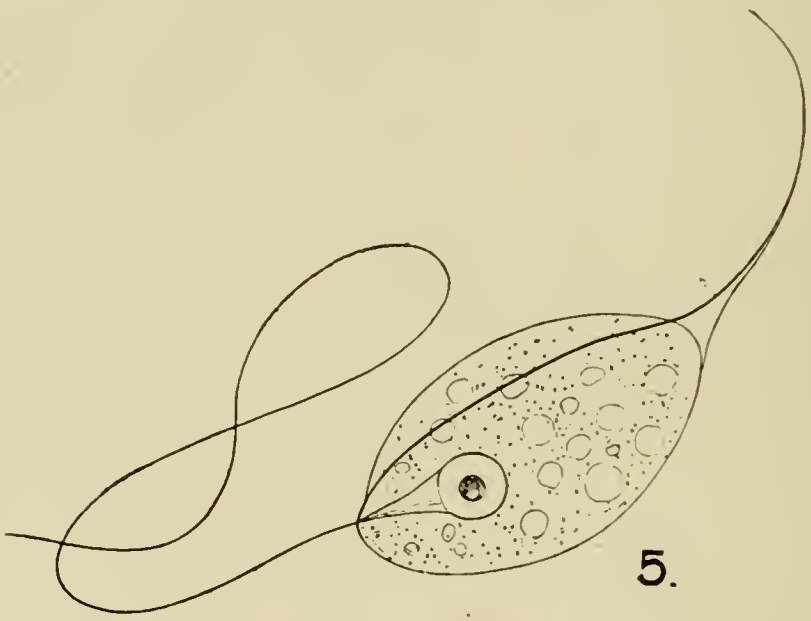
Division-stage of free-swimming form.

the nucleus with the base of the two flagella. The details of these structures are much more evident in the fixed and stained films. The protoplasm of the body contains food and other vacuoles, but contractile vacuole is not present. Sometimes the nucleus is surrounded with refractile granules, having the same greenish line and refraction as the karyosome within the nucleus. These may be present in sufficient numbers as to completely obscure the nucleus. Similar granules occur in the protoplasm of encysted forms (fig. 6). These granules stain deeply, and are possibly of a chromatin nature.

In the hanging-drop preparations especially this organism

exhibited a peculiar polymorphism. In the central part of the hanging drop, where the fluid was deep, the flagellates had the typical pear-shaped appearance, with the long, tapering, posterior extremity. At the sides of the hanging drop, where there was only a thin layer of moisture on the cover-glass, the typical pear shape was lost and the flagellates had the appearance of amœbæ. When first I observed this I thought my culture had become contaminated with an amœba, but the

Text-fig. 5.



Ordinary free-swimming type.

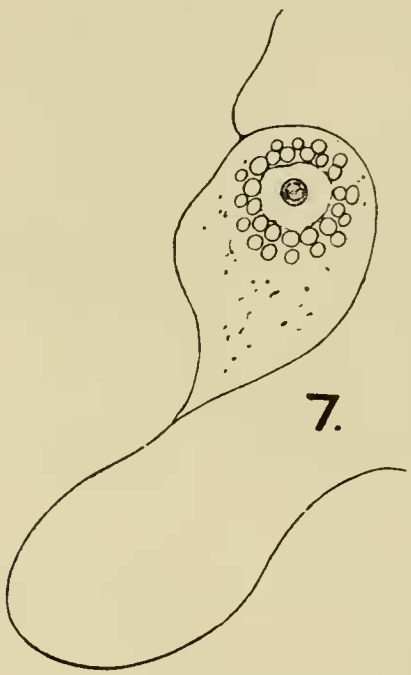
presence of the long anterior flagellum and the short posterior one disproved this idea. It was possible to watch a single individual swimming in the deep part towards the edge. On reaching the shallow part the character of the organism changes at once to the amœboid form. Pseudopodia are protruded and withdrawn, and the animal creeps about in a typical amœboid manner. All this while the long anterior flagellum is lashing to and fro, but appears powerless to draw the animal across the surface of the cover-glass. It is only in the deeper part of the hanging drop that the flagellum is useful. The posterior flagellum is often not visible, and its prolongation across the surface of the body is more difficult to detect.

When seen it is inert and only moves in a passive manner. It seems to take little share in movements of progression.

On the surface of the agar medium the organism is generally of the amœboid form.

At the edge of the hanging-drop preparations or on the surface of the agar it is easy to watch these amœboid forms ingesting food by surrounding objects with pseudopodia. As a rule the amœboid forms contain many more food-vacuoles than those swimming in the deeper layers.

TEXT-FIG. 7.



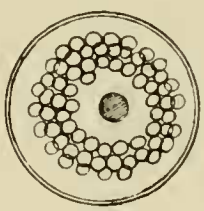
Less regular encysted form.

Reproduction is by longitudinal division. There is first multiplication of the flagella, whether by new formation or division of those already existing has not been determined. The nucleus next divides. The karyosome is divided into two parts, and finally the elongated nuclear membrane becomes constricted and two nuclei are formed. After a short time the protoplasm becomes drawn out and finally a constriction appears, which ultimately ends in complete division. The process of this division is very readily watched on the cover-

glass cultures described above. Both the amœboid and the free-living forms divide in this manner, but on account of the more sluggish movements of the former they are more readily kept under observation.

In the cultures encysted forms commence to appear after a few days. In the liquid cultures they are to be found in the scum on the surface or in the deposit at the bottom. On the agar cultures the cysts appear in the older parts of the culture. On this medium the margin of bacterial growths spreads over the surface, and in this margin the actively reproducing flagellates are to be sought. In the oldest part of the culture no free flagellates can be found, but only the cyst.

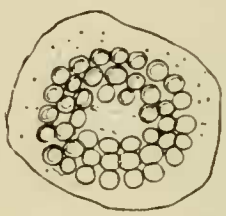
TEXT-FIG. 6.



6.

Encysted forms showing refractile granules surrounding nucleus.

TEXT-FIG. 8.



8.

Free form with refractile granules. Probable preparation for encystment.

In the fresh condition these cysts appear as slightly brownish spherical bodies, with a wall of double contour.

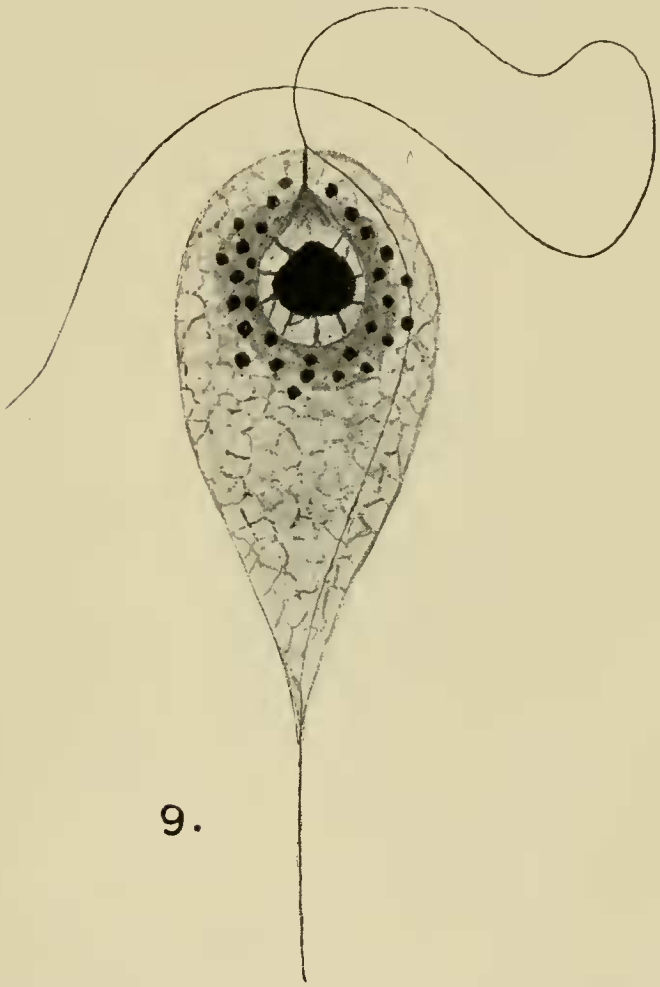
At the centre of the cyst is the spherical nucleus, which has similar characters to that of the free form, except for the prolongation towards the flagella. The nucleus is surrounded by the bright refractile granules, which were described as occurring in some of the free forms. It is probable these granules are of a chromatin nature, and that they arise from chromatin passed out from the nucleus, though this process has not been followed.

Though these organisms have been kept under observation for a year or more conjugation has not been seen, nor has any sexual process been detected. It is possible that some sexual process is bound up with the encystment, but as the

entrance into and emergence from the cyst has not been directly observed and no multiplication within the cyst could be seen nothing definite on this point can be stated.

Text-figs. 9–19.—Drawings from stained preparations.

TEXT-FIG. 9.



Free-swimming form with granules round nucleus.

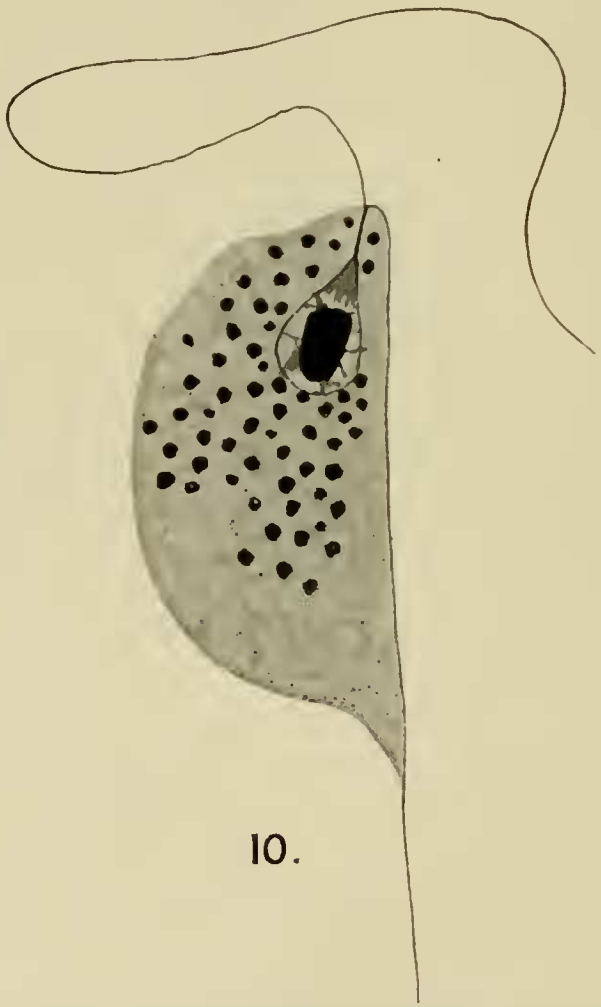
FIXED AND STAINED SPECIMENS.

In the fixed and stained specimens, in addition to the details which were so clearly visible in the living organism, others could be made out.

The protoplasm of the body has a marked alveolar structure. The anteriorly placed nucleus shows a large, deeply staining

karyosome, while connecting this latter body to the nuclear membrane is a coarse linin network. All the chromatin of the nucleus appears to be concentrated in the karyosome. The prolongation of the nuclear membrane towards the

TEXT-FIG. 10.



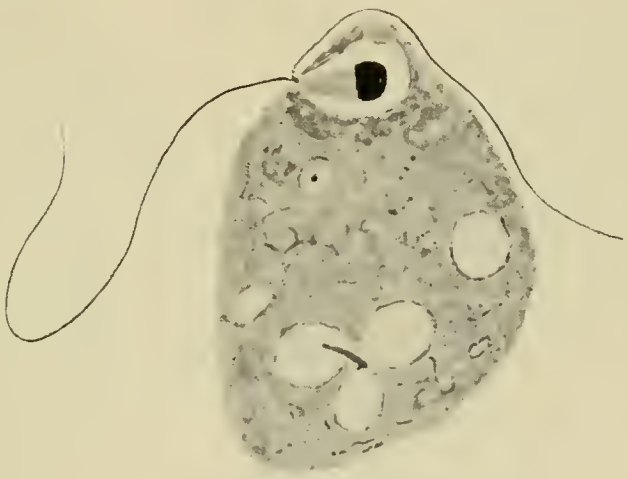
Side view of free form showing the flattened side along which the flagellum runs.

flagella is clearly shown, while the base of these organs is connected to the apex of the nucleus by a rod-like rhizoplast. In some cases the drawn-out apex of the nuclear membrane shows longitudinal markings, which converged toward the rhizoplast, while in others there is a connection in the form of a more deeply staining pyramin between this body and the

karyosome (fig. 11). Prowazek describes for Cercomonas longicaudia a "ein Art undeutlichen Zwischenfibrille," which connects the karyosome to the insertion of the flagella. Prowazek figures this connection as a dark line running from the karyosome to the apex of the nucleus, but I have not been able to detect any structure as definite as the one he figures.

This flagellate is a very excellent illustration of the fallacy of relying for detail on the old dry Romanowsky methods of

TEXT-FIG. 11.



11.

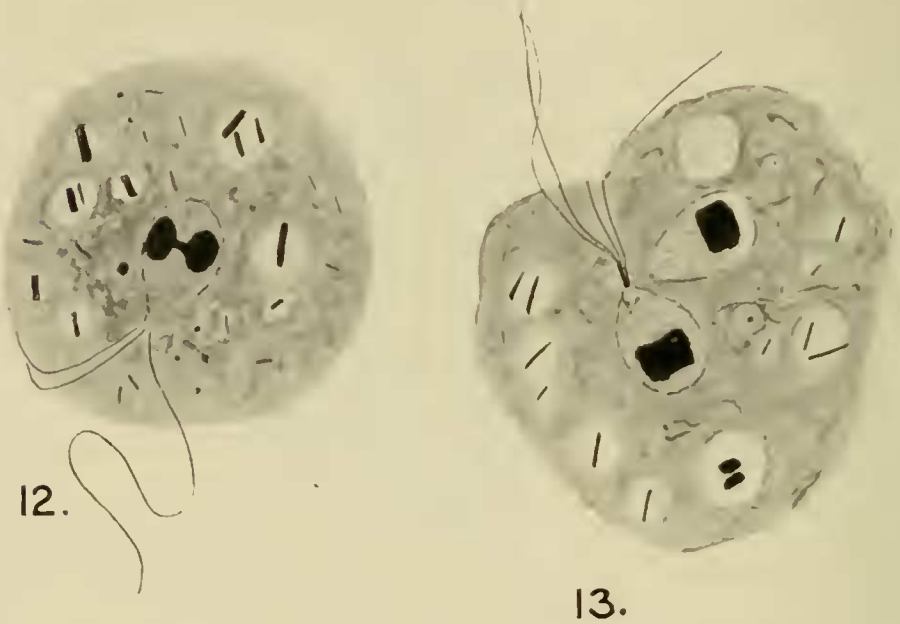
Shows connection of karyosome and rhizoplast.

staining. The nucleus of this organism is clearly visible in the living condition. There is a definite nuclear membrane. At the centre of the nucleus is a large refractile karyosome, while the space between this body and the nuclear membrane is free from granules. The nuclear membrane is drawn out at one point towards the insertion of the flagella. Now if a film of the material containing this flagellate is allowed to dry as in the usual method for the preparation of blood for staining trypanosomes, and stained by one of the modifications of the Romanowsky method, the result may be very beautiful from the colour point of view, but totally misleading in the structure of the nucleus. This latter organ appears in these

dried films as an irregular clump of red staining granules. In other words, its appearances are like those of the nuclei of trypanosomes in similarly prepared films. In films fixed and stained by the wet method described above the structure of the nucleus is comparable with the appearances to be made out in the living organisms.

The details of longitudinal division can be followed in the

TEXT-FIGS. 12, 13.



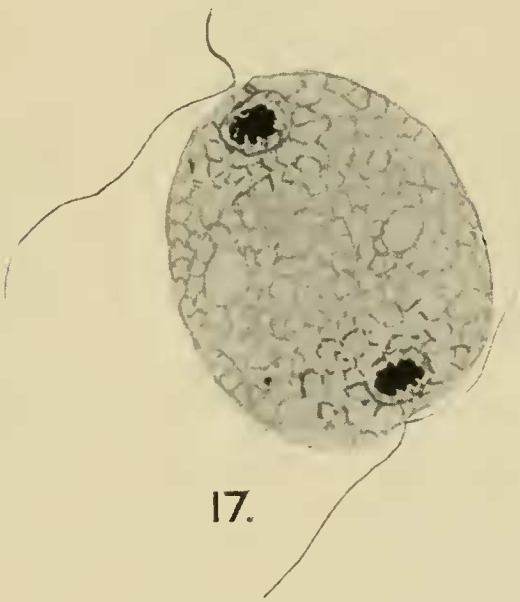
Dividing forms.

stained preparations. The large karyosome becomes elongated and constricted, and finally divided into two parts (fig. 15). I was never able to detect within the karyosome a centriole, spindle, and æquitorial plate, as described by Berliner in the division of Copromonas major, but the division takes place in an amitotic manner, resembling that of Copromonas subtilis (Dobell). Most usually the karyosome becomes distinctly dumb-bell shaped as in fig. 12, but at other times the division is along the longitudinal axis of the elongated karyosome, the resulting daughter-karyosomes each being elongated (figs. 15, 16). Following the division of the karyosome the nuclear membrane elongates while the daughter-

karyosomes separate. The flagella are duplicated at this stage, but they still have a common rhizoplast, which is inserted into one point of the elongated nuclear membrane, which is drawn out slightly at this point towards the anterior end of

TEXT-FIGS. 14-16.

TEXT-FIG. 17.

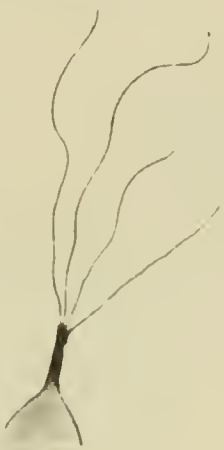


Dividing forms.

the body of the flagellate. Division of the nuclear membrane commences by a constriction at the point opposite the insertion of the rhizoplast. The division is completed, and the two nuclei, each with an apex, are connected to the base of the rhizoplast. The rhizoplast finally divides longitudinally, so that there result two nuclei, each with a rhizoplast and two flagella. The exact method of origin of the flagella I was

unable to trace, though some of the appearances seem to indicate the formation of two new ones by outgrowth from the rhizoplast. In fig. 18 is the nuclear apparatus of a flagellate partially broken up on the film. It shows very clearly the single rhizoplast with the duplicated flagella. The last stage in the division process is thus the splitting of the rhizoplast, while the first stage is the multiplication of the flagella and the commencing division of the karyosome. After complete division the nuclei pass to opposite poles of the body (fig. 17),

Text-fig. 18.



18.

Part of nucleus, rhizoplast, and flagella of partly broken-down individual, to show the multiplication of the flagella before division of the nucleus and rhizoplast.

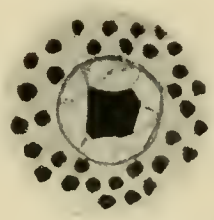
and after a varying interval of time the body is divided into two equal parts.

The bright refractile granules which were described above as occurring in the protoplasm around the nucleus in the encysted forms and in some of the free forms appear in the stained specimens as dark-staining granules. Whether these are chromatin granules of the nature of a chromidium or whether they are capable of some other interpretation cannot be definitely stated, since their fate has not been followed. They certainly stain as chromatin, and their presence within the cyst (fig. 19) would seem to suggest the possibility of

their being nuclei of spores destined to escape from the cyst and ultimately to develop, with or without conjugation, into the adult flagellate form. Though cysts have been constantly kept under observation and every inducement possible to encourage the emergence from the cyst has been tried, I have never been fortunate enough to witness this process. That it does occur is borne out by the experiment of adding dried cysts to fresh medium, resulting in a culture of flagellates.

In the stained preparation certain appearances are capable of interpretation as a conjugation of the flagellates, and some of the nuclear appearances as processes of maturation, but as no undoubted conjugation was observed in the living flagel-

Text-fig. 19.



19.

Cyst showing dark-staining granules surrounding the large central nucleus.

lates I refrain from describing these. Without the control of observation on the living forms descriptions of conjugation and the accompanying nuclear changes are of little value, since the possibility of error in interpretation is very great. For Copromonas major Berliner has described from stained preparations such a process of conjugation, but without the necessary controls it is always possible that abnormal or involution forms have been mistaken for such stages.

In rich cultures of the flagellates there is a very great variation in size. Some individuals are comparatively large, reaching a length of 15 μ or more, excluding the flagella. Others are very minute, being not more than 2–3 μ in longest diameter. All intermediate sizes are to be met with in the cultures. The encysted forms have a diameter of about 6 μ or

7 μ. These cysts will withstand drying at ordinary laboratory temperatures, and are capable of giving rise to fresh cultures when brought into suitable media.

Nomenclature.

It is certain that Dujardin's original description of the genus Cercomonas is incomplete, but it seems to me quite clear from his account that he was dealing with flagellates similar to the one described in this paper. Though he did not definitely state that the fine drawn-out posterior extremity of the body was a flagellum, still, he says that it was at times so fine as to resemble the anterior flagellum, and that it was capable of independent movements. Further, in his table of classification he divides the Monads into two groups. In the first he includes forms with "un seul filament flagelliform," while in the second those with "plusieurs filaments ou appendices." The genus Cercomonas appears in the second of these groups as a form with "un second filament ou appendice postérieur." It is therefore quite evident that Dujardin regarded this posterior termination of the body as of the nature of a flagellum. Stein and Blochmann describe the genus Cercomonas as having a drawn-out posterior end, though they do not describe a definite flagellum. The genus Cercomonas was not accurately defined by Kent or Bütschli, and to Klebs the confusion seemed so great that he proposed the rejection of this generic name and the substitution of Gruber's name Dimorpha, which was created for a bi-flagellate showing at certain stages definite heliozooid characters. In this genus Dimorpha Klebs included forms which he identified with those described originally by Dujardin as Cercomonas, and he suggests that this observer has overlooked the second flagellum. We have seen how near Dujardin was to definitely describing this second flagellum, so that the action of Klebs in rejecting this genus is hardly sound. It seems to me clear that the forms described by Dujardin really possessed two flagella, though

he failed to see this clearly. On this account I think it safer to retain the genus Cercomonas for flagellates of the character described in this paper, viz. flagellates with an anterior blunt end from which arises a single long flagellum and a posterior tapering end also with a flagellum, traceable over the surface of the body towards the insertion of the anterior flagellum. This conclusion is come to by Prowazek also, who figures Cercomonas longicauda with two flagella arising from the nucleus.

The specific name of this flagellate is difficult to determine. Dujardin named several species of Cercomonas, though he was careful to state that he was far from regarding these as true species, but as a convenient means of distinguishing the forms met with in different infusions. From the figures of Dujardin and Stein it is possible that the flagellate belongs to the species longicauda, so that the flagellate described here may be assumed to be Cercomonas longicauda Dujardin.

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